Buechler et al. Application No.: 09/453,234 Page 2

Please replace the paragraph beginning on page 43, line 6, with the

-

PATENT Buechler et al. Application No.: 09/453,234 Page 3 TT ACC CCT GTG GCA AAA GCC CAG GTG CAG CTA CAG CAG TGG GG 947 (SEQ ID NO:18) Please replace the paragraph beginning on page 44, line 38, with the following $\eta \in$ amended paragraph: (SEO ID NO:19) GAC AGA TGG TGC AGC CAC AGT 953 Please replace the paragraph beginning on page 47, line 17, with the following amended paragraph: 5' ATC TGG CAC ATC ATA TGG ATA AGT TTC GTG TAC AAA ATG CCA GAC CTA GAG Re-GAA TTT TAT TTC CAG CTT GGT CCC (SEQ ID NO:20) Please replace the paragraph beginning on page 47, line 22,: with the following amended paragraph 5' GTG ATG GTG ATG GTG ATG GAT CGG AGT ACC AGG TTA TCG AGC CCT CGA TAT TGA GGA GAC GGT GAC TGA (SEQ ID NO:21) Please replace the paragraph beginning on page 47, line 35, with the following amended paragraph: Primer 5 B7 5' GCA ACT GTT GGG AAG GG (SEQ ID NO:22) Please replace the paragraph beginning on page 47, line 38, with the following amended paragraph: B8 Primer 197 5' TC GCT GCC CAA CCA GCC ATG (SEQ ID NO:23) Please replace the paragraph beginning on page 48, line 8, with the following amended paragraph: 5' PCR primer (869)- GGG ACC AAG CTG GAA ATA AAA CGG GCT GTG GCT GCA CCA TCT B9

Please replace the paragraph beginning on page 48, line 11, with the following

3' PCR primer (870)- ATC TGG CAC ATC ATA TGG ATA AGA CTC TCC CCT GTT GAA GCT

GTC T (SEQ ID NO:24)

amended paragraph:

CTT (SEQ ID NO:25)

R10

PATENT

Buechler et al. Application No.: 09/453,234 Page 4

Please replace the paragraph on page 48, line 14, with the following amended

paragraph:

5' PCR primer (867)- TCA GTC ACC GTC TCC TCA GCC TCC ACC AAG GGC CCA TC (SEQ ID NO:26)

Please replace the paragraph on page 48, line 16, with the following amended

paragraph:

BIZ 3' PCR primer (876)- GTG ATG GTG ATG GTG ATG AGA TTT GGG CTC TGC TTT CTT GTC C

Please replace the paragraph on page 50, line 14, with the following amended

paragraph:

B13 Primer 885 5' TAA GAG CGG TAA GAG TGC CAG (SEQ ID NO:27)

> Please replace the paragraph beginning on page 64, line 6, with the following amended paragraph:

The polyclonal IL8 antibody phage form both the 109 and 1010 affinity cuts (see Example 13) were diluted 1/30 in 2 x YT and 1 μ l used as template for PCR amplification of the antibody gene inserts with primers 197 (Example 5) and 970 (see below). PCR (3-100 µL reactions) was performed using a high-fidelity PCR system, Expand (Roche Molecular Biochemicals, Indianapolis, IN) to minimize errors incorporated into the DNA product. Each 100 µl reaction contained 100 pmol of 5' primer 197, 100 pmol of 3' primer 970, 0.7 units of Expand DNA polymerase, 10 µl 2 mM dNTPs, 10 µl 10 x Expand reaction buffer, 1 µl diluted phage stock as template, and water to 100 µl. The reaction was carried out in a Perkin-Elmer thermal cycler (Model 9600) using the following thermal profile: one cycle of denaturation at 94 °C (1 min); ten cycles of denaturation (15 sec, 94 °C), annealing (30 sec, 55 °C), elongation (60 sec, 72 °C); fifteen cycles of denaturation (15 sec, 94 °C), annealing (30 sec, 55 °C), elongation (80 sec plus 20 sec for each additional cycle, 72 °C); elongation (6 min, 72 °C); soak (4 °C, indefinitely). The PCR products were ethanol precipitated, pelleted and dried as described above. The DNA was dissolved in water and fractionated by agarose gel electrophoresis. Only full-length products were excised from the gel, purified, and resuspended in water as described earlier.

 $\mathcal{B}^{\mathfrak{n}}$

Buechler et al. Application No.: 09/453,234

Page 5

B14

Primer 970- 5' GT GAT AAA CTA CCG TA AAG CTT ATC GAT GAT AAG CTG TCA A TTA GTG ATG GTG ATG GTG ATG AGA TTT G (SEQ ID NO:29)

Please replace the paragraph beginning on page 67, line 15, with the following amended paragraph:

The decapeptide, YPYDVPDYAS (SEQ ID NO:30), (Chiron Mimotopes Peptide Systems, San Diego, CA) was dissolved (0.3 g) in dry DMF (5.4 mL) in a round bottom flask under argon with moderate stirring. Imidazole (0.02 g) was added to the stirring solution. Separately, acetylthiopropionic acid (0.041 g) was dissolved in 0.55 mL of dry DMF in a round bottom flask with stirring and 0.056 g of 1,1'-carbonyldiimidazole (Aldrich Chemical Co., Milwaukee, WI) was added to the stirring solution. The flask was sealed under argon and stirred for at least 30 min at room temperature. This solution was added to the decapeptide solution and the reaction mixture was stirred for at least six hr at room temperature before the solvent was removed in vacuo. The residue in the flask was triturated twice using 10 mL of diethyl ether each time and the ether was decanted. Methylene chloride (20 mL) was added to the residue in the flask and the solid was scraped from the flask and filtered using a fine fritted Buchner funnel. The solid was washed with an additional 20 mL of methylene chloride and the Buchner funnel was dried under vacuum. In order to hydrolyze the derivative to generate a free thiol, it was dissolved in 70% DMF and 1 M potassium hydroxide was added to a final concentration of 0.2 M while mixing vigorously. The derivative solution was allowed to stand for 5 min at room temperature prior to neutralization of the solution by the addition of a solution containing 0.5 M potassium phosphate, 0.1 M borate, pH 7.0, to which concentrated hydrochloric acid has been added to a final concentration of 1 M. The thiol concentration of the hydrolyzed decapeptide derivative was determined by diluting 10 μL of the solution into 990 μL of a solution containing 0.25 mM 5,5'-dithiobis(2nitrobenzoic acid) (DTNB, Aldrich Chemical Co., Milwaukee WI) and 0.2 M potassium borate, pH 8.0. The thiol concentration in mM units was equal to the A412(100/13.76).

B15

-	A 71	מידי	TT
Ρ	Δ 1	Hr	N I
	~~ 1	1	4 T

1 .	Buechler et al.	<u>PATENT</u>
1	Application No.: 09/453,234 Page 6	
	Please replace the paragraph beginning on page 72, line 12, with the following	
	amended paragraph:	
	A- 5' (TCGCTGCCCAACCAGCCATGGCCAGTGCTAAAGAACTTAGATCTCAG)	1
1 (_	(SEQ ID NO:31) B- 5' (GTGATAAACTACCGCATTAAAGCTTATCGATGATAAGCTGTCAATTAGT	GAT
8,6	GGTGATGGTGAATTCTCAGCCCTCTTCAA) (SEQ ID NO:32	
	C- 5'(GCAACTCTCTACTGTTTCTCC) (SEQ ID NO:33) D- 5'(GAGGATGACGATGAGCGC) (SEQ ID NO:34)	7
	D- 5' (GAGGATGACGATGAGCGC) (SEQ ID NO.34)	
	Please replace the paragraph on page 75, line 18, with the following amended	1
- R 17	paragraph: M1-1L (SEQ ID NO:35)	
1-15	M1-1L (SEQ 1D NO:35)	
	Please replace the paragraph on page 76, line 4, with the following amended	
	paragraph:	
R18	M1-3L (SEQ ID NO:37)	
	MI-3B (SBQ 12 Ac.3.)	
	Please replace the paragraph on page 76, line 17, with the following amended	!
	paragraph:	
BIT	M1-4L (SEQ ID NO:39)	
1	Please replace the paragraph on page 76, line 30, with the following amended	!
	paragraph:	
R 20	M1-5L (SEQ ID NO:41)	
		!
1	Please replace the paragraph on page 76, line 43, with the following amended	!
	paragraph:	
B21	M1-8L (SEQ ID NO:43)	
	The state of the s	!
1	Please replace the paragraph on page 76, line 56, with the following amended	Ī
	paragraph:	
B22	M1-10L (SEQ ID NO:45)	
l	The Rewitter following amended	
1	Please replace the paragraph on page 77, line 8, with the following amended	
- , २	paragraph:	
B23	M1-21L (SEQ ID NO:47)	
4		

n		т	,,,	. A	7
Р	- 4		Έ	1	
_	4 1		_	Τ,	

,	Buechler et al. Application No.: 09/453,234 Page 7	<u>PATENT</u>
	Please replace the paragraph on page 77, line 19, with the following amended	
	paragraph:	
B 24	M1-23L (SEQ ID NO:49)	
B25	Please replace the paragraph on page 77, line 34, with the following amended paragraph:	
<i>D</i>	M1-25L (SEQ ID NO:51)	
	Please replace the paragraph on page 77, line 47, with the following amended paragraph:	
B26	M1-1H (SEQ ID NO:53)	
	Please replace the paragraph on page 77, line 60, with the following amended paragraph:	
B 21	M1-3H (SEQ ID NO:55)	
	Please replace the paragraph on page 78, line 12, with the following amended paragraph:	
B 28	M1-4H (SEQ ID NO:57)	
	Please replace the paragraph on page 78, line 25, with the following amended paragraph:	
B29	M1-5H (SEQ ID NO:59)	
	Please replace the paragraph on page 78, line 38, with the following amended paragraph:	
B30	M1-8H (SEQ ID NO:61)	
	Please replace the paragraph on page 78, line 51, with the following amended paragraph:	
D31	M1-10H (SEQ ID NO:63)	
U	MI-ION (DBQ ID NO.03)	
	Please replace the paragraph on page 79, line 4, with the following amended paragraph:	
0.32	M1-21H (SEQ ID NO:65)	
_B	EL ZIII (DIQ ID IO. CO)	

, .	Buechler et al.	PATENT
	Application No.: 09/453,234 Page 8	
	Please replace the paragraph on page 79, line 17, with the following amended	
_	paragraph:	
803	M1-23H (SEQ ID NO:67)	
	Please replace the paragraph on page 79, line 30, with the following amended	
	paragraph:	
B34	M1-25H (SEQ ID NO:69)	
:	Please replace the paragraph on page 79, line 43, with the following amended	
	paragraph:	
B3.5	M2-11L (SEQ ID NO:71)	
2		
	Please replace the paragraph on page 79, line 56, with the following amended	
L,	paragraph:	
B3c	M2-12L (SEQ ID NO:73)	
	Please replace the paragraph on page 80, line 8, with the following amended	
	paragraph:	
B ³⁷	M1-16L (SEQ ID NO:75)	
	Please replace the paragraph on page 80, line 21, with the following amended	
B38	paragraph:	
. 6	M2-18L (SEQ ID NO:77)	
	Please replace the paragraph on page 80, line 34, with the following amended	
	paragraph:	
B ³⁹	M2-20L (SEQ ID NO:79)	
	Please replace the paragraph on page 80, line 47, with the following amended	
	paragraph:	
B40	M2-31L (SEQ ID NO:81)	
	Please replace the paragraph on page 80, line 60, with the following amended	
	paragraph:	
B41	M2-32L (SEQ ID NO:83)	

P	1	١,	T	Ē	N	IT

· .	Buechler et al. Application No.: 09/453,234 Page 9	<u>PATENT</u>
	Please replace the paragraph on page 81, line 12, with the following amended	
	paragraph:	
B42	M2-33L (SEQ ID NO:85)	
	Please replace the paragraph on page 81, line 25, with the following amended paragraph:	
B43	M2-34L (SEQ ID NO:87)	
	Please replace the paragraph on page 81, line 38, with the following amended paragraph:	
R44	M2-35L (SEQ ID NO:89)	
	Please replace the paragraph on page 81, line 51, with the following amended paragraph:	
045	M2-11H (SEQ ID NO:91)	
. 13 '	Please replace the paragraph on page 82, line 4, with the following amended	
	paragraph:	
B46	M2-12H (SEQ ID NO:93)	
	Please replace the paragraph on page 82, line 17, with the following amended paragraph:	
B47	M2-16H (SEQ ID NO:95)	
	Please replace the paragraph on page 82, line 30, with the following amended	
~ 48	paragraph:	
B48	M2-18H (SEQ ID NO:97)	
	Please replace the paragraph on page 82, line 43, with the following amended paragraph:	
B49	рагаgrapн. M2-20H (SEO ID NO:99)	
	M2-200 1980 ID NO.331	
	Please replace the paragraph on page 82, line 56, with the following amended	
1365	paragraph:	
B30	M2-31H (SEQ ID NO:101)	

1 1 1 1 1 1 1

,	Buechler et al. Application No.: 09/453,234 Page 10	PATENT
	Please replace the paragraph on page 83, line 8, with the following amended	
	paragraph:	
B31	M2-32H (SEQ ID NO:103)	
	Please replace the paragraph on page 83, line 22, with the following amended	
B52	paragraph: M2-33H (SEQ ID NO:105)	
<u> </u>	MZ-33H (SEQ 15 NO.103)	
	Please replace the paragraph on page 83, line 35, with the following amended paragraph:	
B=3	M2-34H (SEQ ID NO:107)	
	Please replace the paragraph on page 83, line 48, with the following amended	
	paragraph:	
Bry	M2-35H (SEQ ID NO:109)	
	Please replace the paragraph beginning on page 84, line 3, with the following	
Been	amended paragraph: Translated amino acid sequences of sequenced antibodies. M1-H Heavy Chain Variable and CH1 Regions 10 ⁻⁹ M ⁻¹ Affinity Cut (SEQ ID NOS:64,54,66,68,70,56,58,60 and 62 respectively)	
	Please replace the paragraph beginning on page 84, line 61, with the following amended paragraph:	
B36	M1-L Kappa Chain Variable and Constant Regions 10 ⁻⁹ M ⁻¹ Affinity Cut (SEQ ID NOS:46,36,48,50,52,38,40,42, and 44 respectively)	
	Please replace the paragraph beginning on page 85, line 56, with the following amended paragraph:	
BST	M2-H Heavy Chain VH-CH1 Sequence 10 ⁻¹⁰ M ⁻¹ Affinity Cut (SEQ ID NOS:92 94, 96, 98, 100, 102, 104, 106, 108, and 110 respectively)	,
	Please replace the paragraph beginning on page 86, line 57, with the following amended paragraph:	
0 ==	M2-L Kappa Chain VKCK 10 ⁻¹⁰ M ⁻¹ Affinity Cut (Thu Sep 23) (SEQ ID NOS:72, 74	1 ,
355	76, 78, 80, 82, 84, 86, 88, and 90 respectively) .	